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Evaluation of a Baculovirus Bioinsecticide for Small-Scale Maize Growers in Latin America

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Near identical trials conducted concurrently in maize plots in Honduras and Mexico produced similar results in the pattern of larval mortality of *Spodoptera frugiperda* following application of a baculovirus or a conventional synthetic insecticide. The highest application rates of virus resulted in approximately 40% mortality of *S. frugiperda* larvae. Virus-induced mortality decreased with time. Parasitism by wasps and tachinids also contributed up to 40% mortality in field-collected larvae. The application of chlorpyrifos resulted in a resurgence of *S. frugiperda*. Chlorpyrifos also reduced a number of important predators in the maize crop which is likely to have been influential in the observed resurgence of this pest. The use of granulated sugar in the viral formulation caused an increase in the population density of several maize-associated insect species, and in Mexico a transient increase in parasitism was observed in sugar-treated plots. Sugar did not appear to increase the probability of infection by acting as a feeding stimulant in either trial. A preliminary analysis of the cost of viral production and application indicates that virus was considerably more costly than conventional control. To be commercially viable, economies of scale both in the cost of raw material for the insect diet and in the efficiency of manpower-related activities are needed to substantially reduce the costs of the viral product. Despite high levels of infestation by *S. frugiperda*, grain weight/cob was not significantly improved by the application of the biological or synthetic insecticide. Natural mortality factors both biotic and abiotic appear to have a large impact on larval *S. frugiperda* populations. For improvements in yield, the impact of control measures against *S. frugiperda* may be dependent on plant growth stage. Trials on timing and frequency of virus application are in progress to test this idea.

Key Words: **nucleopolyhedrovirus; *Spodoptera frugiperda;* fall armyworm; biological control; microbial control; corn pest.**

INTRODUCTION

Small-scale producers of maize in Latin America require simple, cheap and sustainable methods of production (Andrews *et al.*, 1992). The principal economic input in maize production relates to control of the major pest, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). This insect may account for a 20% reduction in yield or substantially more in certain situations (Andrews, 1988; Hruska and Gould, 1997). To control infestation by *S. frugiperda* and stem borers, mainly *Diatraea lineolata* (Walker), small-scale maize growers in Mexico and Central America typically use methyl parathion, chlorpyrifos, methamidophos, or some other readily available synthetic insecticide, applied using a backpack sprayer or as granules applied by hand. In addition to the increased costs of production, clear detrimental effects on the health of farm workers arising from the extensive use of such chemicals have been observed in rural communities in Latin America (McConnell and Hruska, 1993; Tinoco and Halperin, 1998).

Various attempts have been made to evaluate the potential of natural enemies of *S. frugiperda* for biological control of the pest, including parasitoids (Yassen *et al.*, 1981; Cave, 1993; Carrillo-Sánchez, 1993; also reviewed by Andrews, 1988), predators (Van Huis, 1981; Jones *et al.*, 1989), and microbial agents (Agudelo-Silva, 1986; Fuxa, 1982). Although predators and parasitoids can be important mortality factors, mass rearing and liberation are not usually considered as a viable option due to the low commercial value of maize.

The principal feeding damage caused by this pest occurs to the leaves within the developing whorl although larvae may attack seedlings as cutworms. Consequently, the economic threshold for chemical control of *S. frugiperda* is reasonably high; typically around 15–30% infestation, which appears to make the pest

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suitable for integrated control practices that do not have an instantaneous effect (Van Huis, 1981).

Baculoviruses, applied as bioinsecticides, have shown considerable promise both for large-scale production (Young and Hamm, 1966; Hamm and Young, 1971) and small-scale, low technology situations (Jones *et al.*, 1993). The use of such viruses has a number of advantages over conventional measures, in that they are host specific, simple to apply, amenable to a variety of formulations, compatible with the action of other natural enemies, and safe to humans (Entwistle and Evans, 1985). One limitation to the use of baculoviruses has been their relatively slow speed of kill, but this is not a serious drawback for crops like maize that can withstand moderate defoliation without significant losses in yield or market value.

The present study focuses on the use of a nucleopolyhedrovirus of *S. frugiperda* (SfNPV), originally isolated in Nicaragua and recently characterized (Escribano *et al.*, 1999). This study represents part of a project to develop a sustainable system of pest control for small-scale maize growers in Latin America and as such seeks to identify simple practices that permit maize growers to farm without the need for costly chemical inputs. Here, we report the results of field trials performed in Mexico and Honduras evaluating the effect of viral formulations and application rates on crop yields and the prevalence of maize pests and associated natural enemies.

MATERIALS AND METHODS

The climatic and geographical characteristics of each experimental area are as follows. The Mexican trial was performed on the grounds of CIICA (Centro Internacional de Investigación y Capacitación Agropecuaria), Frontera Hidalgo, 18 km south-east of Tapachula on the coastal plain of Chiapas and 1 km from the border with Guatemala, at an altitude of approximately 50 m above sea level. The climate during the months of May to November is warm and humid (typical daily temperature ranges from 35°C maximum to 23°C minimum) with a mean monthly rainfall of 300 mm and a relative humidity of >85% during the growing season. The Honduran trial was performed in the valley of El Zamorano, 30 km southeast of Tegucigalpa, 825 m above sea level, with a daily temperature range of 20–30°C, a relative humidity exceeding 75%, and approximately 200 mm of rainfall per month during May to November.

Independent cultures of locally collected *S. frugiperda* were maintained on semisynthetic diet at ambient laboratory temperatures in El Colegio de la Frontera Sur (ECOSUR), Mexico and the Escuela Agrícola Panamericana (EAP), El Zamorano, Honduras. Third-stage larvae from these cultures were used to produce the Nicaraguan SfNPV isolate. Viral-killed larvae were stored at −10°C until required.

To extract polyhedral inclusion bodies (PIBs), larvae were thawed, liquidized in 0.1% SDS, and centrifuged at 90*g* for 5 min and the supernatant was centrifuged at 3000*g* for 10 min. Pelleted PIBs were resuspended in distilled water and quantified using a bacterial counting chamber. This suspension was stored at 4°C for no longer than 48 h prior to use in the field trial.

Field Trial, Mexico

A multifactorial field trial was planned to test the effect of viral formulation and application rate on *S. frugiperda* infestation and crop yield. A locally common variety of maize (Tacsa-H101) was planted in 36 blocks of 5 × 5 m at a standard density of 25 cm between plants and 70 cm between rows (Andrews, 1980). A gap of 5 m between blocks was planted with 2 rows of maize to reduce interplot movement of arthropods and help buffer plots from storm damage. The trial was performed in June 1997 during the rainy season (May–November) when maize is normally planted.

Preliminary formulation tests with sand or sawdust indicated that neither substance was a suitable carrier because the virus bound strongly to both and was not readily released by sequential washes that imitated the action of rainfall. Previous observations in Honduras had indicated that sugar may attract natural enemies (ants, parasitoids, coccinellids, etc.) (Cañas and O'Neil, 1998) and might also act as a feeding stimulant for *S. frugiperda*, increasing the probability of larvae consuming a lethal dose of virus (R. Trabanino, pers. comm.). Ordinary refined sugar from a local supermarket was therefore selected as the basis for the granular formulation. One day prior to the field trial, between 111 µl and 2.22 ml of viral suspension (depending on the application rate) was added to 1.23 kg of granular sugar, followed by very thorough mixing for a minimum of 10 min.

At 26 days after planting, maize plants were 35–45 cm tall with eight to nine leaves (midwhorl stage). At this time plots were infested with approximately 450 recently emerged first-stage larvae (the equivalent of four egg masses/plot). Larvae were distributed randomly within each plot by walking through the plot slowly releasing larvae contained within a paper bag. Two subsequent liberations of larvae occurred at weekly intervals (33 days and 40 days after planting) at a rate of 250 larvae/plot, each 4 days before an evaluation point.

At 28 days post planting, virus was applied as a spray including wetter-sticker (0.2% Agralplus, Zeneca) or as 2 g of sugar applied directly into the whorl. Sugar was applied using a plastic teaspoon which represented a uniform dosing method, with a low variability (2.04 ± 0.017 g, mean ± SE, $n = 30$).

For each formulation, three application rates were used: 50, 250, or 1000 larval equivalents (LE)/ha, assuming that 1 LE was equivalent to 6×10^9 PIBs (Young and Hamm, 1966; R. Cave pers. obs.). The volume of spray applied to each plant was on average 14.5 ml, most of which was directed into the whorl. A typical backpack sprayer was used for all liquid applications. A standard insecticide treatment consisting of chlorpyrifos (Lorsban 480EM) applied as a spray at the recommended rate of 1 liter/ha was included in the trial. Treatments were randomly assigned to plots and there were four replicate plots per treatment.

Two days after viral application, an evaluation was made involving dissection of 20 plants/plot. Plants were selected by reference to random number tables. Each plant was allocated a damage score on a 0–4 scale (0 = 0–10%; 1 = 11–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% defoliation) (Kaya *et al.*, 1995).

The presence of all observed arthropods was recorded using standardized data sheets. In general, broad groupings were used to classify insects and spiders, e.g., predatory bugs (mainly *Orius* spp.), ants (mostly *Solenopsis* spp.), all types of spider, *Chrysoperla* spp. (all stages), predatory Coleoptera (coccinelids, carabids, and staphylinids), dipteran larvae (mainly syrphids), colonies of aphids (comprising a minimum of 20 individuals, lesser infestations of aphids were ignored), other pests (leafhoppers, etc.), or other natural enemies (adult parasitoids, etc.). When the identity of an insect was uncertain, it was taken to the laboratory for identification.

Any *S. frugiperda* larvae found were transferred to semisynthetic diet and reared in the laboratory until pupation. Causes of death in these larvae were noted daily and any emerging parasitoids were identified. Viral infection was diagnosed by observation of inclusion bodies in a Giemsa-stained smear of insect tissues. Plants tasseled earlier than expected and only two additional evaluations were made at weekly intervals (9 and 16 days) following viral application, giving a total of three sample time points.

The grain weight/cob from each treatment was determined as the weight of grain from 30 cobs/plot. Cobs were harvested at 124 days after planting when the average moisture content was 13.08%, and grain weights were subsequently corrected for moisture content on an individual plot basis. The planting density and shelling efficiency (grain wt/[grain wt + cob wt]) were the same for all plots, meaning that grain weight/cob should reflect differences in yield among treatments.

Field Trial, Honduras

The trial in Honduras was identical to that in Mexico, except in the following respects. *S. frugiperda* larvae were liberated into field plots of maize variety Hb-104 as early second instars 4 days prior to evaluation. The virus spray was formulated with 0.06% Adsee (Westrade Guatemala S.A.) as a wetter-sticker rather than Agral-plus. Only the presence of *S. frugiperda* larvae was recorded in each experimental plot. A total of four post-application samples were made at 2, 9, 16, and 23 days post-application. Yield was assessed as the weight of grain from 60 cobs/plot which was harvested 115 days after planting with a 13.1% moisture content.

Statistical Analysis

All analyses were performed using GLIM (Generalized Linear Interactive Modeling). Analyses of virus and parasitoid (but not insecticide) induced *S. frugiperda* mortality were performed using raw data and a binomial error structure resulting in deviance changes that approximate to a χ^2 distribution or were scaled and are given as F values (Crawley, 1993). Numbers of insects recovered and damage scores in treatment plots were analyzed using Poisson errors and were scaled where necessary. Viral application rate was analyzed as a continuous variable; the alternative analysis as a discrete variable with 4 levels (0, 50 LE, 250 LE, and 1000 LE/ha) made little difference to the outcome of treatment effects. Grain weight was analyzed using a normal error distribution. Data from each sample in each trial were analyzed independently of one another. In all cases examination of residuals was used to check the accuracy and behavior of the models.

Cost Analysis

The costs of producing the virus were calculated without taking into account the setup costs of equipping the laboratory, training personnel, etc. or commercial aspects such as storage, distribution, and marketing. The overhead costs are also small in comparison with those of more developed regions. Nevertheless, this analysis reflects the running costs of producing the virus in a working laboratory in the region.

RESULTS

Viral Mortality, Parasitism, and Changes in Pest Density

Mortality of *S. frugiperda* in field-collected larvae reared on diet in the laboratory was positively related to viral application rate in Mexico ($\chi^2{}_1 = 19.13$, $P < 0.001$ for larvae collected at 2 days post-application and $\chi^2{}_1 = 5.00$, $P < 0.05$ for larvae collected at 9 days post-application) and Honduras ($F_{1,32} = 12.00$, $P < 0.01$ for larvae collected at 2 days; $\chi^2{}_1 = 6.83$, $P < 0.05$ for larvae collected at 9 days; $F_{1,32} = 5.53$, $P < 0.05$ for larvae collected at 15 days post-application). In both trials, the proportion of virus-infected larvae observed in field samples decreased over time (Figs. 1 and 2). In the larvae reared from the sample at 2 days post-application, the highest prevalence of viral disease was observed in the 1000 LE/ha plots at 39.6% in Mexico (Fig. 1A) and 37.8% in Honduras (Fig. 2A), but for the larvae reared from the samples collected at 15 to 23 days post-application, virus accounted for very little *S. frugiperda* mortality in Honduras or none at all in Mexico (Figs. 1B, 1C, and 2B–2D). Formulation had no significant effect on the prevalence of virus-induced mortality in field-collected larvae at any timepoint in either Mexico or Honduras.

The natural background level of *S. frugiperda* infestation was high in the Honduran trial, approximately

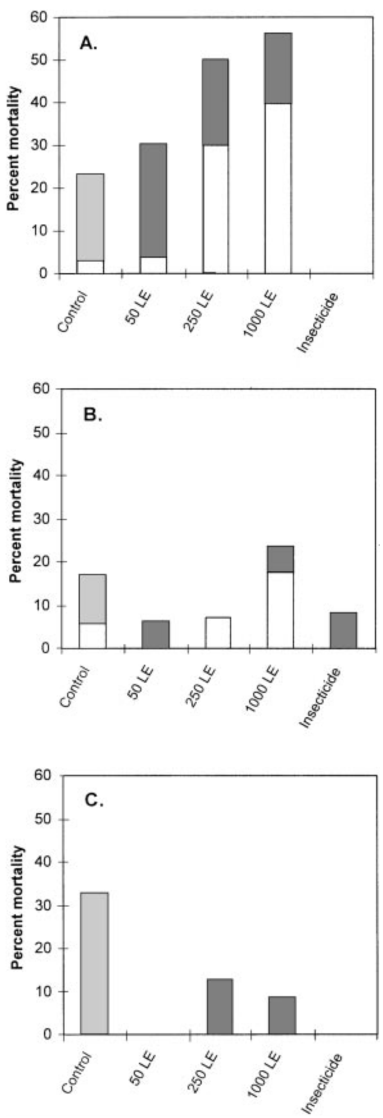

FIG. 1. Percentage viral mortality and parasitism in *Spodoptera frugiperda* larvae collected and reared on semisynthetic diet until death or pupation (spray and sugar formulations combined). Larvae collected at (A) 2 days, (B) 9 days, and (C) 15 days post-application in the Mexican trial. Virus-induced mortality ▭; parasitism ■. LE, larval equivalents.

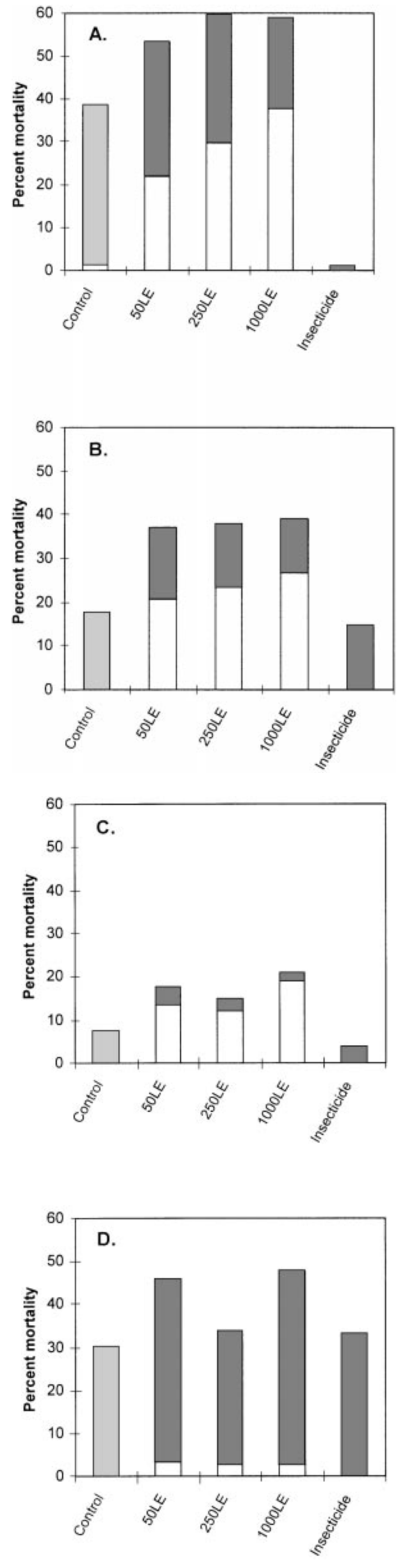

FIG. 2. Percentage viral mortality and parasitism in *Spodoptera frugiperda* larvae collected and reared on semisynthetic diet until death or pupation (spray and sugar formulations combined). Larvae collected at (A) 2 days, (B) 9 days, (C) 15 days, and (D) 23 days post-application in the Honduran trial. Virus-induced mortality ▭; parasitism ■. LE, larval equivalents.

50% compared to the Mexican counterpart (11.5%, random sample of 400 plants). This infestation level was enhanced by liberation of *S. frugiperda* larvae such that by the first sample, infestation levels of 0.8–1.2 larvae/plant were observed in Honduras compared to 0.3–0.4 larvae/plant in Mexico (based on plots not treated with chlorpyrifos) (Fig. 3). The density of *S. frugiperda* in control and treated plots fell steadily during the experimental period except where chlorpyrifos was applied (discussed below).

Parasitism by wasps and tachinids also contributed substantially to mortality in recovered larvae. The most commonly observed parasitoids were *Chelonus insularis* Cresson (Braconidae), *Eiphosoma vitticolle* Cresson (Ichneumonidae), *Ophion flavidus* Brullé (Ichneumonidae), *Cotesia marginiventris* (Cresson) (Braconidae), *Archytas marmoratus* (Townsend) (Tachinidae), and *Lespesia archippivora* (Riley) (Tachinidae), all of which are commonly found attacking *S. frugiperda* in Central America (Cave, 1995).

Levels of parasitism were generally higher in the Honduran trial than in Mexico (Fig. 1 and 2). The application of sugar enhanced the prevalence of parasitism at the 2-day post-application sample in Mexico ($\chi^2_1 = 6.31$, $P < 0.01$), but not at subsequent sample periods. No significant effects of formulation were observed in Honduras at any sampling period. There was no obvious interaction between the presence of virus and the probability of parasitism in either trial. Parasitism and virus-induced disease combined to cause a maximum *S. frugiperda* mortality of nearly 60% at the 2-day post-application sample in each trial (Figs. 1A and 2A).

Treatment by chlorpyrifos caused markedly different

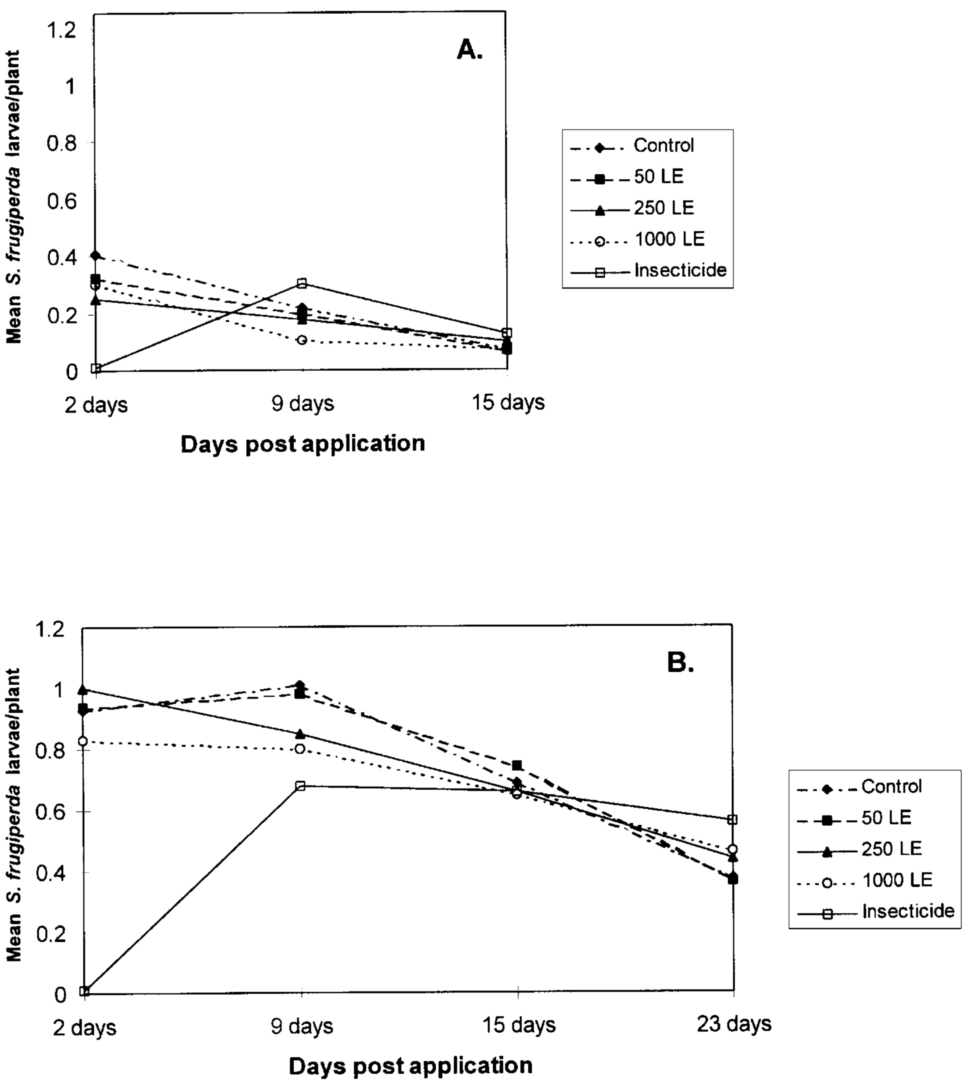

FIG. 3. Changes in the density of *Spodoptera frugiperda* larvae in experimental plots during the period of the trial (A) in Mexico and (B) in Honduras (data from each formulation combined). LE, larval equivalents.

patterns of mortality. In both trials, insecticide application reduced *S. frugiperda* recovery to virtually zero at 2 days post-application but subsequent reinfestation resulted in a *S. frugiperda* density some 37% greater than in control plots at 15 days post-application (Fig. 3). This density fell at the third sample but remained more than 60% higher than untreated controls. In the Honduran trial, chlorpyrifos resulted in a marked reduction in levels of parasitism at 2 days post-application ($\chi^2_1 = 28.1$, $P < 0.001$) but by the following sample period, 9 days post-application, the prevalence of parasitism was statistically similar to that observed in other treatments (Fig. 2B).

Comparison of *Eco*RI and *Hin*dIII restriction endonuclease profiles of virus from field-killed insects with profiles of virus stock inoculum indicated no discernible differences between samples for a random selection of 10 larvae (data not shown). This strongly suggests that the virus applied in the field was the cause of observed *S. frugiperda* mortality, rather than provoking the activation of a widespread latent infection of experimental insects.

Effects on Other Maize Arthropods

Due to logistical limitations, analysis of arthropods present in the experimental samples was undertaken only in the Mexican trial. At 2 days post-application, chlorpyrifos reduced the numbers of predatory earwigs, mostly *Doru taeniatum* (Dohrn) ($\chi^2_1 = 14.90$, $P < 0.001$), *Diatraea* spp. ($\chi^2_1 = 3.84$, $P < 0.05$), aphids ($\chi^2_1 = 4.04$, $P < 0.05$), beetles ($\chi^2_1 = 7.79$, $P < 0.01$), and predatory bugs (mainly *Orius* spp.) ($\chi^2_1 = 4.42$, $P < 0.05$) (Fig. 4A), but thereafter had no significant effect on the presence of maize-associated arthropods with the exception that at 15 days post-application, the presence of *Phyllophaga* spp. was very significantly lower in chlorpyrifos-treated plots ($\chi^2_1 = 66.85$, $P < 0.001$) (Fig. 4C).

Formulation also produced significant changes in the presence of certain other insects. At 2 days post-application, sugar-treated plots had higher levels of aphids ($\chi^2_1 = 9.60$, $P < 0.01$), Coleoptera ($\chi^2_1 = 18.52$, $P < 0.001$), and dipteran larvae ($\chi^2_1 = 4.81$, $P < 0.05$), and a reduced density of *Chrysoperla* spp. ($\chi^2_1 = 12.07$, $P < 0.001$) (Fig. 4A), whereas at 9 days post-application, sugar-treated plots had more dipteran larvae ($\chi^2_1 = 6.69$, $P < 0.01$) and ants ($\chi^2_1 = 5.17$, $P < 0.05$) but fewer earwigs ($\chi^2_1 = 7.69$, $P < 0.01$) and fewer predatory bugs ($\chi^2_1 = 5.27$, $P < 0.05$) than plots treated with the spray formulation of virus (Fig. 4B).

Effects on Foliar Damage and Grain Weight

Analysis of the visual damage to plants indicated that at 2 days post-application plants had less damage in chlorpyrifos-treated plots ($\chi^2_1 = 9.2$, $P < 0.01$) (data not shown). Formulation was also significant; visual damage scores were 17% lower in sugar-treated plots ($\chi^2_1 = 10.11$, $P < 0.01$). These effects were temporary, however, as by 9 or 15 days post-application no significant differences in damage levels among treatments were detected.

In the Mexican trial, grain weight per cob (following correction for moisture content) was not significantly affected by any treatment, although application rate of virus was very nearly significant ($\chi^2_1 = 3.95$, $P = 0.055$), with a steady increase in yield with concentration (Table 1). Cob grain weight from chlorpyrifos-treated plots was virtually identical to the controls. In comparison, in the Honduran trial grain weights were generally greater and more variable among replicate plots, but no significant treatment effects were detected.

Cost Analysis

The cost of producing and applying the virus in each country was calculated following the details given in Table 2. In Mexico, the total cost for producing and applying virus at a rate of 1000 LE/ha was estimated at US $48.43 compared to US $34.98 in Honduras, a difference mainly attributable to higher labor costs in Mexico. This contrasts with the cost of 1 liter of chlorpyrifos per hectare which in Mexico costs US $15.74 and in Honduras costs US $12.25 at current exchange rates. In Mexico, the virus is produced as an experimental product for local testing and evaluation, whereas the virus is produced on a small semi-commercial scale in Honduras.

DISCUSSION

Twin trials in Honduras and Mexico using near identical experimental protocols produced similar results in terms of the pattern of mortality achieved following application of a baculovirus specific to *S. frugiperda* or a conventional synthetic insecticide. The highest application rates of virus resulted in approximately 40% mortality of *S. frugiperda* recovered from experimental plots. Virus-induced mortality decreased with time. The reasons why greater levels of viral disease were not achieved may relate to the persistence of the inoculum in the field. Following application in the early morning, virus was subject to intense solar radiation for the remainder of the day. The susceptibility of baculoviruses to UV light is well recognized as an important limitation to their use as bioinsecticides (Ignoffo, 1992). Early morning rather than dusk application was chosen as being more typical of conventional growing practices in both regions.

Viral inoculum may also be rapidly diluted by the fast growth of the maize plant such that within a short period, little or none of the inoculum remains in the feeding area of the pest. Moreover, heavy rain falling every day in the mid-afternoon during the rainy season

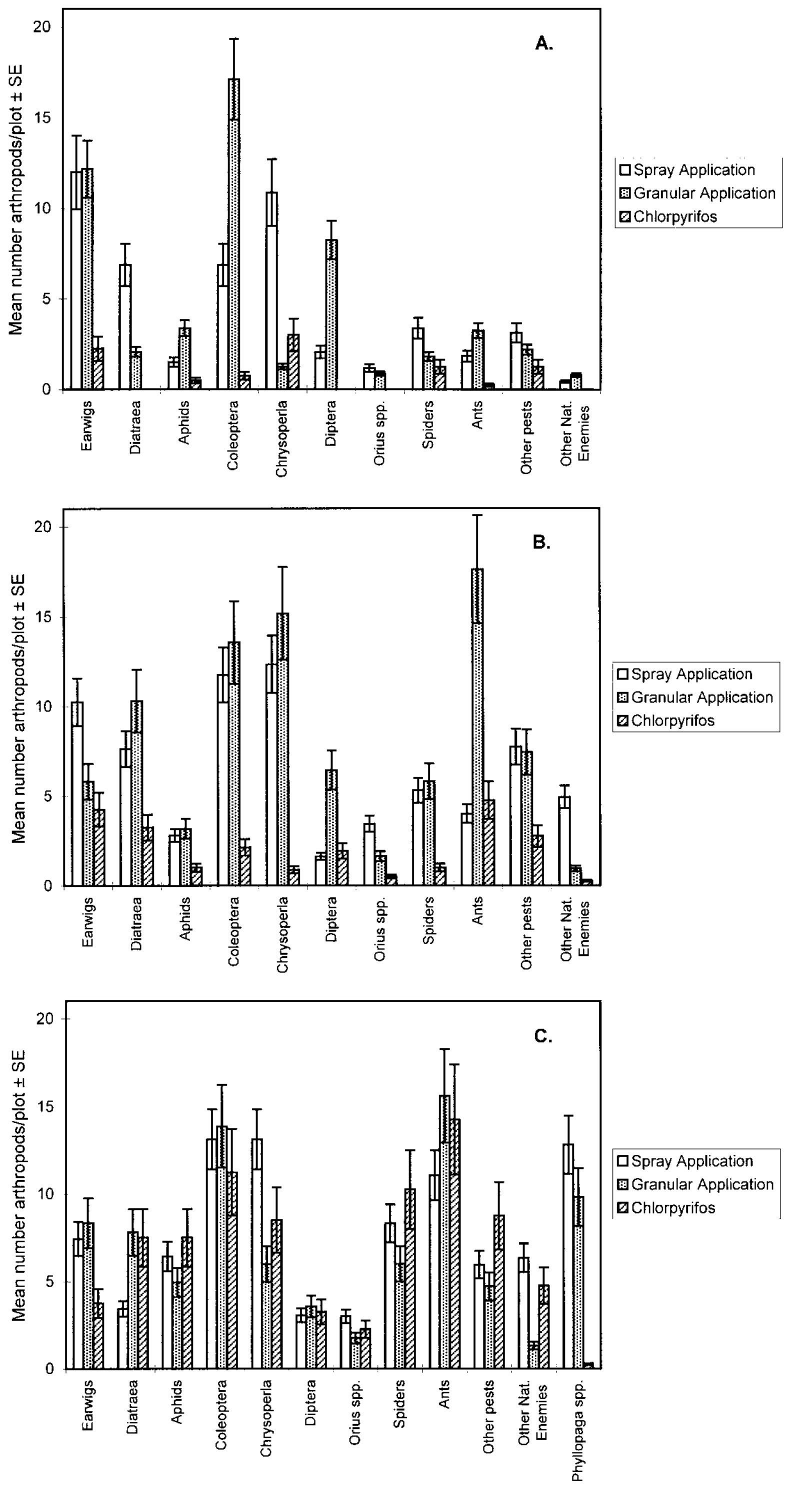

FIG. 4. Effect of viral formulation or chlorpyrifos application on the presence of maize-associated arthropods in Mexican field trial at (A) 2 days, (B) 9 days, and (C) 15 days post-application. Bars are mean number (±SE) arthropods per plot (per 20 plants sampled) (see text for details of significant differences).

TABLE 1

Grain Weight per Cob for *Spodoptera frugiperda* Nucleopolyhedrovirus [Expressed in Larval Equivalents (LE)] Spray and Granule Formulations Combined, Expressed as Mean ± SE of 30 (Mexico) or 60 (Honduras) Cobs Sampled per Plot (in grams, Dry Weight)

	Control	Chlorpyrifos	50 LE	250 LE	1000 LE
In Mexico	105.1 ± 2.98	105.2 ± 1.69	103.0 ± 2.81	104.2 ± 2.92	110.6 ± 2.81
In Honduras	99.4 ± 4.18	116.3 ± 6.94	117.1 ± 6.23	112.9 ± 7.17	102.8 ± 4.89

may wash away inoculum to exterior leaf surfaces or to the soil. Following egg hatch, *S. frugiperda* larvae move immediately to the uppermost leaves and the whorl where they stay for their entire larval development (Labatte, 1993) and are therefore unlikely to become infected by viral inoculum contaminating foliage outside the whorl.

The application of chlorpyrifos had a detrimental impact on parasitism and resulted in a resurgence of *S. frugiperda*, clearly observed in both trials. Chlorpyrifos also reduced the numbers of important predators in the maize crop, notably earwigs, *Orius* spp., and predatory beetles, which is likely to have been influential in the observed resurgence of *S. frugiperda*.

The use of granulated sugar as a formulation agent increased the numbers of several insects but only transiently enhanced parasitism levels (only observed in Mexico), whereas previous observations had suggested that sugar applied as a spray could enhance the densities of both parasitoids and predators within the maize crop (Cañas and O'Neil, 1998). Sugar also did not appear to increase the probability of infection by acting as a feeding stimulant in either trial.

The preliminary analysis of the cost of virus production and application indicates that virus is considerably more costly than conventional control. The analysis assumes, however, that virus would be applied at a rate of 1000 LE/ha. If one or two applications at a lower rate were applied, e.g., 250 LE/ha, the costs of control would be around US $13.00 to US $26.00/ha, favorably comparable to the cost of chlorpyrifos. These figures are preliminary because in both laboratories the virus is produced in relatively small quantities. Economies of scale in the cost of raw material for the insect diet etc. and in the efficiency of manpower-related activities are expected to substantially reduce the costs of viral product. The analysis does not include unquantifiable factors such as the risk to human health and environmental impact of using synthetic insecticide.

TABLE 2

Breakdown of Costs Associated with Producing and Applying *Spodoptera frugiperda* Nucleopolyhedrovirus at 1000 Larval Equivalents (LE) of Virus in Mexico and Honduras

Activity/Materials	Mexico	Honduras
Manpower (man-hours) to maintain insect culture, prepare diet, wash and clean, to infect and harvest 1000 larvae	13.0	13.6
Salary costs, technician/h	US $2.54	US $1.16
Total labor costs	US $33.02	US $15.78
Materials for 1 liter of diet	US $0.98	US $1.25
Number of larvae reared to adulthood/liter of diet	115	112
Diet costs for 1000 larvae	US $8.48	US $13.37
Purification and counting of virus (materials & labor costs for 2 h)	US $5.67	US $3.75
Wetter-sticker for 1 hectare	US $1.26	US $2.08
Total cost for control over 1 hectare (1000 LE)	US $48.43	US $34.98

Economic threshold recommendations for *S. frugiperda* vary from 11 to 40% of plants infested (Andrews and Rueda, 1986; Evans and Stansly, 1990), although the majority have fallen in the 20–30% range (Sarmiento and Casanova, 1975; Van Huis, 1981). In our study, despite high levels of infestation, grain weight/cob was not significantly improved by the application of the biological or the synthetic insecticide. It appears that natural mortality factors, both biotic and abiotic, have a large impact on larval *S. frugiperda* populations. The ability of maize plants to withstand defoliation could also be significant such that in the mid-whorl stage, control measures against *S. frugiperda* may not lead to improvements in yield. In a detailed study of the effects of insect attack on maize production in Central America, Hruska and Gould (1997) reported that plants were more tolerant to insect attack during early growth stages than at later stages. When 55–100% of plants were infested by *S. frugiperda*, yield losses of 15–73% were observed. There was also a high degree of correlation between infestation with *S. frugiperda* and attack by *D. lineolata*. When the two pests co-occurred, economic injury thresholds varied from 23 to 63% infestation.

In several respects, our study has raised more questions than it has answered, both with regard to the limitations of the virus as a biological control agent and for the importance of control measures directed against *S. frugiperda*. Preliminary observations have indicated that, compared to the early season infestations studied in the present trial, mid/late season control of *S.*

frugiperda may be unnecessary due to high densities of natural enemies.

Few studies have attempted to reproduce near identical experimental procedures for simultaneous evaluation of control measures in different developing regions, as achieved in our study. The high degree of similarity of results among the two field experiments is reassuring in terms of the reliability of our findings. Additional trials are currently in progress to determine the effect of repeated applications of virus at a range of doses and the importance of timing of control measures in relation to plant growth stage.

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